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Table of Contents

INTRODUCTION

CBP and p300 are highly related mammalian transcriptional coactivators that regulate gene transcription through various activities (Goodman and Smolik, 2000). Both coactivators are known to enhance gene transcription by linking sequence-specific transcription factors to the RNA polymerase II holoenzyme (Kee et al., 1996; Nakajima et al., 1997). They also promote gene transcription by forcing chromatin into conformations that are more accessible to DNA binding transcription factors through the acetylation of histones (Ogryzko et al., 1996). Besides histones, CBP and p300 also acetylate specific transcription factors. For instance, acetylation of p53 by CBP has been shown to enhance the DNA binding ability of p53 (Gu et al., 1997; Liu et al., 1999; Sakaguchi et al., 1998), and acetylation of CREB has been shown to stimulate CREB-mediated gene expression (Lu et al., 2003).

Mice lacking CBP or p300, or that are heterozygous for both CBP and p300, typically die around day 10.5 of embryogenesis with severe developmental retardation, reduced size, defects in heart development, and lack of neural tube closure (Oike et al., 1999; Yao et al., 1998). Although this confirms the notion that CBP and p300 are functionally conserved paralogs, specific differences in phenotype between the CBP and p300 null embryos suggest that these coactivators also have non-redundant functions. For example, only CBP null embryos exhibit defects in primitive hematopoiesis (Oike et al., 1999; Yao et al., 1998). CBP heterozygous mice, but not p300 heterozygous mice, show craniofacial and skeletal defects that are reminiscent of Rubinstein-Taybi syndrome (RTS) (Yao et al., 1998), a human disorder caused by mono-allelic mutations in the CBP gene (Petrij et al., 1995). Furthermore, CBP heterozygous mice show reduced self-renewal capacity of hematopoietic stem cells, while p300 heterozygous mice do not (Rebel et al., 2002). Lastly, mice with point mutations in the p300 KIX domain that disrupt the binding surface for the transcription factors c-Myb and CREB, show severe hematopoietic abnormalities including anemia, B-cell deficiency, thymic hypoplasia, megakaryocytosis and thrombocytosis (Kasper et al., 2002). By contrast, age-matched mice with identical mutations in the CBP KIX domain appear essentially normal.

Several lines of evidence suggest the involvement of CBP and p300 in tumor formation. CBP and p300 are at the breakpoints of several chromosomal translocations in human leukemias (reviewed in Blobel, 2000; Goodman and Smolik, 2000), and provide coactivator functions to translocation generated fusion proteins such as NUP98-HOXA9 and MOZ-TIF2 (Deguchi et al., 2003; Kasper et al., 1999). CBP and p300 have also been implicated in the actions of viral oncoproteins

such as human T-cell leukemia virus 1 (HTLV-1) Tax protein, adenovirus E1A protein, simian virus (SV) 40 T antigen and human papillomavirus (HPV) E6 (reviewed in Goodman and Smolik, 2000). Mutations of p300 have been found in several epithelial cancers, including breast and colon. In some of these tumors, loss of heterozygosity has been detected, suggesting that p300 may act as a tumor suppressor (Gayther et al., 2000; Muhua et al., 1998). Mutations of p300 and CBP have been found in several epithelial cancers, including breast and colon. In some of these tumors, loss of heterozygosity has been detected, suggesting that p300/CBP may act as a tumor suppressor.

The **central hypothesis** of our proposal is that loss of CBP or p300 function in breast epithelium will result in an increased frequency of cell transformation through deregulation of proto-oncogenes and tumor-suppressor genes required for proper control of cell proliferation and differentiation.

The main **purpose** of the research project is to determine, by the use of a conditional knockout approach, whether CBP and p300 exert tumor suppression activity in the breast, and if so, to identify cancer-critical genes whose expression is altered following the loss of CBP and may participate in CBP/p300-mediated mammary gland tumorigenesis.

BODY

The studies that we performed in year 01 concentrated on specific aim 1, the goal of which is to determine whether CBP and p300 exert tumor suppression activity in mouse mammary gland tissue by the use of CBP and p300 conditional knockout mouse models.

We intercrossed $CBP^{loxP/loxP}$ mice and MMTV-Cre transgenic mice to generate mice without CBP in mammary glands. We obtained MMTV-Cre$CBP^{flox/flox}$ mice. These mice were viable and displayed no overt phenotypes during post-natal development. We employed various methods, including indirect immuno-fluorescence and Southern and Western blot analysis, to determine that CBP efficiently disrupted in mammary gland epithelial cells of MMTV-Cre$CBP^{flox/flox}$ mice. As expected, CBP was efficiently disrupted in these cells. We also observed various levels of disruption in other tissues, including skin, thymus, salivary gland, spleen and bone marrow (Figure 1). We analyzed the effect of CBP disruption of mammary gland development

by comparing the histology of MMTV-Cre $CBP^{loxP/loxP}$ and $CBP^{loxP/loxP}$ female mice during puberty (at 4 and 12 weeks of age), pregnancy, lactation and post-lactation. This analysis revealed that MMTV-Cre$CBP^{flox/flox}$ mice are fertile, produce normal litter sizes and are able to nurse their pups. Mammary gland development appeared normal during puberty, pregnancy, and the lactation and post-lactation phases.

To determine whether MMTV-Cre$CBP^{flox/flox}$ mice were predisposed to mammary gland tumors, we generated a cohort of 40 MMTV-Cre $CBP^{loxP/loxP}$ females (experimental group) as well as 19 $CBP^{loxP/loxP}$ females (control group), and monitored these mice for tumor formation or ill health. To our surprise, we found that fifty-nine percent of these mice developed fatal T cell lymphomas between 3 and 9 months of age. Histological analysis of tumor sections typically showed a monotonous infiltrate of lymphoblasts with numerous mitotically active cells. Extensive lymphoblast infiltration generally occurred in non-lymphoid and lymphoid organs including lung, liver, kidney, spleen and lymph nodes. Immunohistochemical and Western blot analysis of lymphoma tissue from six mice demonstrated a complete lack of CBP protein in each of the tumors (Figure 2). As expected, expression of p300 appeared normal. Twenty-nine MMTV-Cre$CBP^{flox/flox}$ mice stayed healthy during the observation period of 10 months, as did all mice $CBP^{flox/flox}$ control groups. Analysis of the thymuses of two long-term surviving MMTV-Cre$CBP^{flox/flox}$ mice for Cre-recombination showed a lack of CBP in 96 and 51% of thymocytes, respectively, confirming that their CBP disruption frequency is similar to that of the mice that develop tumors.

Of the MMTV-Cre$CBP^{flox/flox}$ mice that stayed lymphoma-free, 5 died for unknown reasons, 7 mice have developed life-threatening mammary tumors and 4 are have remained healthy (see Figure 3). In contrast, 3 out of 19 Cre$CBP^{flox/flox}$ control females died for unknown reasons, 2 developed mammary tumors and 14 have remained healthy. By the use of Southern blot analysis and immuno-histochemistry, we confirmed that each of the tumors from MMTV-Cre$CBP^{flox/flox}$ mice were CBP deficient. The macroscopic appearance of each of the larger tumors was always nodular (Figure 3B). All tumors were histologically confirmed by a Mayo Clinic pathologist. **From these findings, we conclude that CBP is a genuine tumor suppressor gene in mouse mammary glands.** We are currently continuing to monitor the remaining mice for tumors or ill health to finalize this tumor susceptibility study. We have collected tumor tissue for studies that we proposed in specific aim 2, the goal of which is to identify target genes that are deregulated by the loss of CBP and test

whether these deregulations might play a key role in the development of CBP-mediated mammary gland tumorigenesis.

Furthermore, we have been successful in obtaining p300 conditional knockout mice. We have started to intercross $p300^{loxP/loxP}$ mice with MMTV-Cre transgenic mice to disrupt p300 in mammary gland epithelium. We plan to generate a group of 60 MMTV-Cre $p300^{loxP/loxP}$ and 60 $p300^{loxP/loxP}$ females.

KEY RESEARCH ACCOMPLISHMENTS

- The demonstration that CBP is not essential for mammary gland development and function.
- The demonstration that CBP is a genuine tumor suppressor gene in mouse mammary glands.
- The collection of mammary gland tumors that lack CBP for the gene expression studies proposed in the second aim.
- The successful generation of p300 conditional knockout mice allowing for comparitive analysis of the tumor suppressive function of CBP and p300 in mammary glands.

REPORTABLE OUTCOMES

The generation of CBP conditional knockout mice and the monitoring of these mice for tumor formation spans roughly a 2-year period. Therefore, there have been no reportable outcomes in the first year of funding.

CONCLUSIONS

The main conclusion of our work is that CBP is a genuine tumor suppressor in the mammary gland of the mouse. This result suggests that CBP is likely to prevent the development breast cancer in human. Our work also indicates that CBP is a likely target for mutation in breast cancers. This result prompted us to start collaborating with Dr. Fergus Couch to screen 200 breast cancers from Mayo Clinic patients for mutations in the CBP gene locus.

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APPENDICES

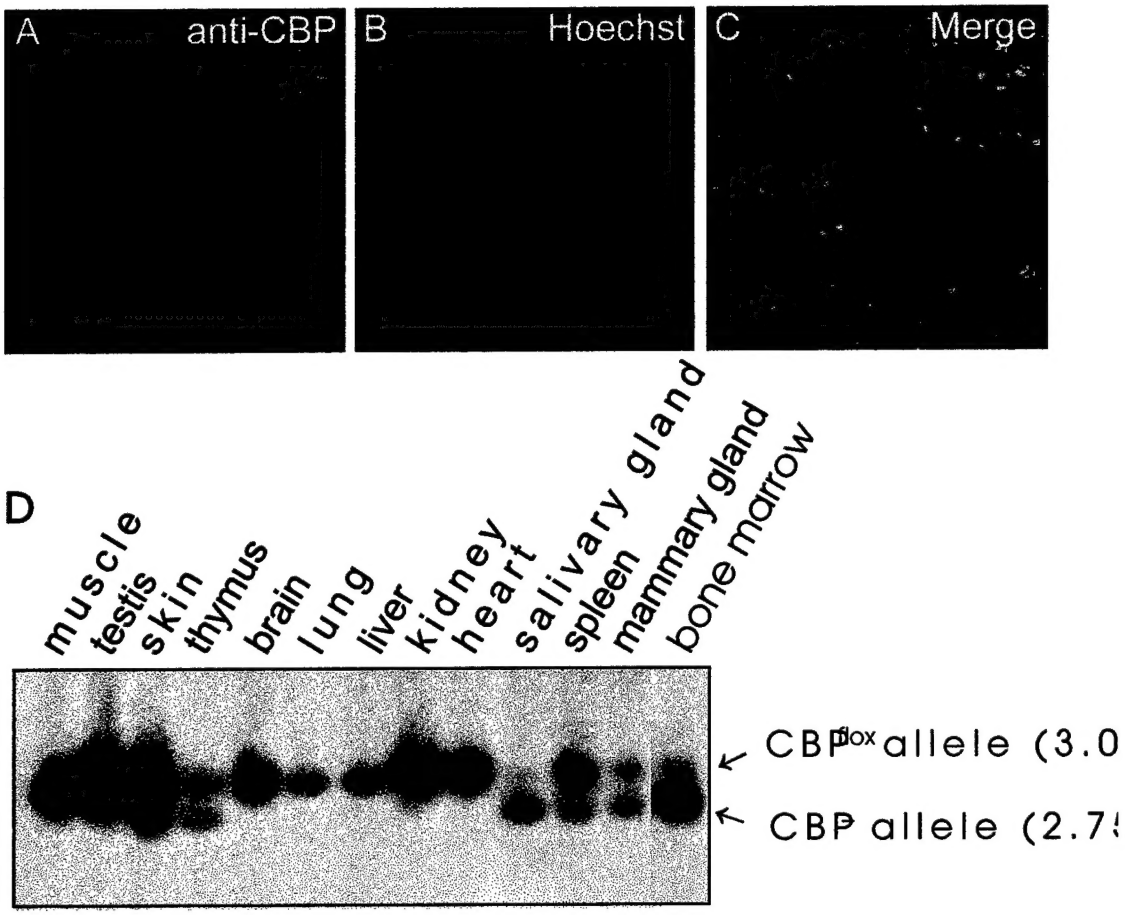


Figure 1:
(A-C) Immunohistochemical staining of mammary gland section from a MMTV-Cre|CBP$^{flox/flox}$ mouse with antibody against CBP. Note that virtually all epithelial cells lack CBP staining. (D) Southern blot analysis of EcoRI-digested DNA from various tissues of an 8-week-old MMTV-Cre|CBP$^{flox/flox}$ female mouse (the testis sample was from a male mouse of the same genotype). The sizes of the bands representing the CBP^{flox} and CBP^{-} alleles are indicated.

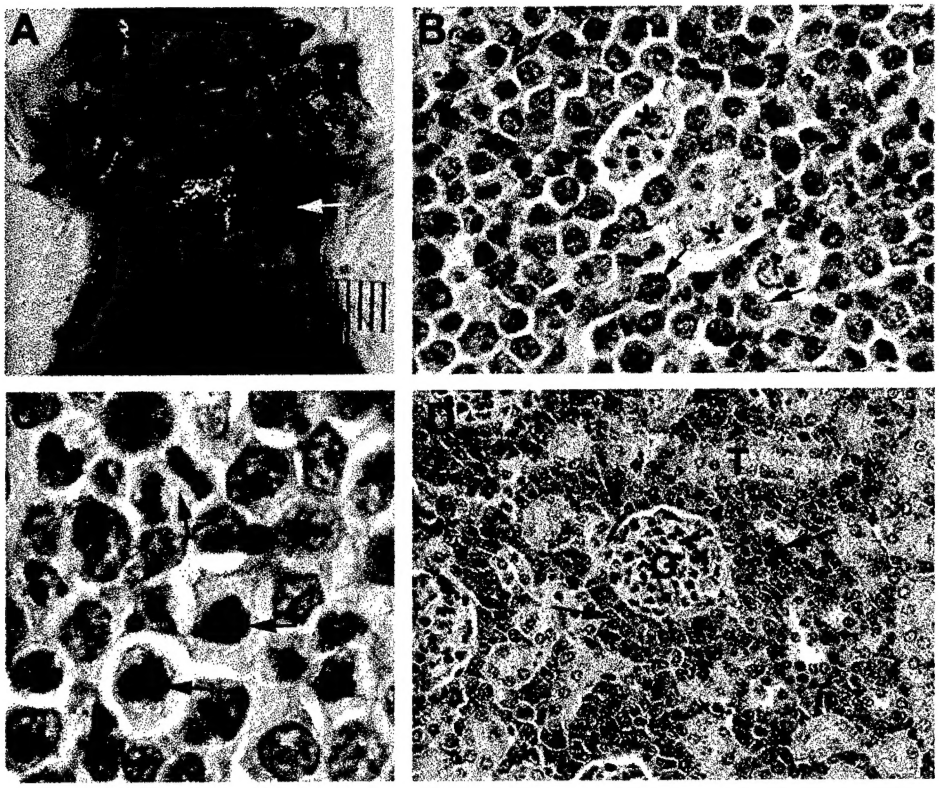
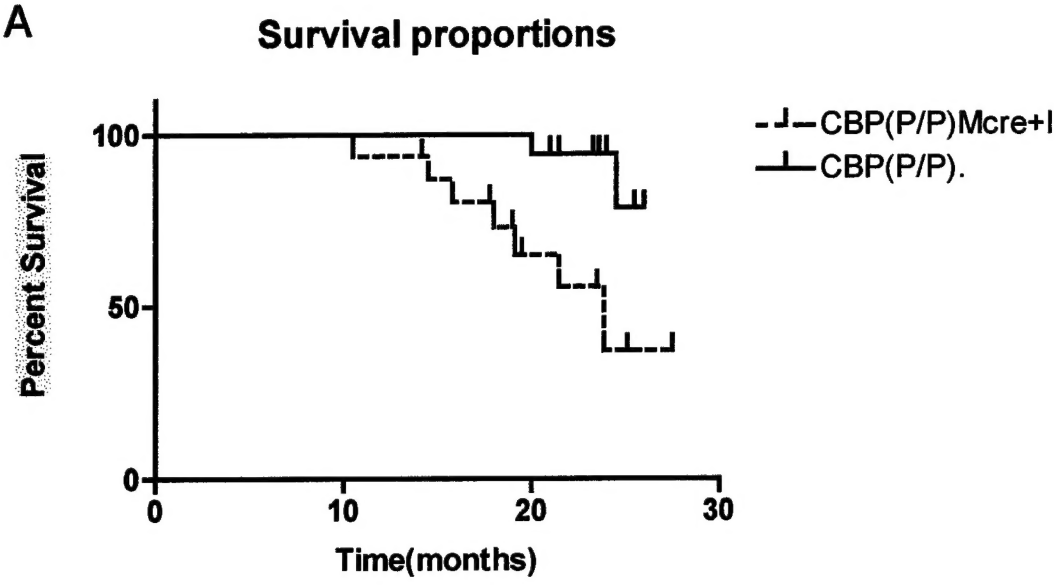


Figure 2:

(A) Gross morphology of a representative T cell lymphoma (arrow). **(B-D)** Histopathology of tumors from moribund MMTV-Cre|CBP$^{flox/flox}$ mice (hematoxylin and eosin-stained paraffin sections). (B) Large macrophages with nuclear debris of lymphocytes in their cytoplasm (asterisks) embedded in uniform populations of highly mitotic thymic lymphoblasts (arrows). (C) Detail of mitotic lymphoblasts (arrows). (D) Lymphoma cells (arrows) infiltrated in kidney tissue (abbreviations: G marks a glomerulus and a renal tubule).

A



B

Figure 3:
(A) Mammary gland tumor incidence in MMTV-Cre|CBP$^{flox/flox}$ among the cohort of females that did not develop thymic lymphoma. Mice that died but were not confirmed by necropsy to have any tumors were excluded from the data presented. **(B)** Gross morphology of a representative mammary tumor (arrow).